

# Beet Leafhopper (Hemiptera: Cicadellidae) Transmits the Columbia Basin Potato Purple Top Phytoplasma to Potatoes, Beets, and Weeds

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**ABSTRACT** Experiments were conducted to determine whether the beet leafhopper, *Circulifer tenellus* (Baker) (Hemiptera: Cicadellidae), transmits the purple top phytoplasma to potato, *Solanum tuberosum* L.; beets, *Beta vulgaris* L.; and selected weed hosts. The beet leafhopper-transmitted virescence agent (BLTVA) phytoplasma was identified as the causal agent of the potato purple top disease outbreaks that recently occurred in the Columbia Basin of Washington and Oregon. The phytoplasma previously was found to be associated almost exclusively with the beet leafhopper, suggesting that this insect is the probable vector of BLTVA in this important potato-growing region. Eight potato cultivars, including 'Russet Burbank', 'Ranger Russet', 'Shepody', 'Umatilla Russet', 'Atlantic', 'FL-1879', 'FL-1867', and 'FL-1833', were exposed for a week to BLTVA-infected beet leafhoppers. After exposure, the plants were maintained outdoors in large cages and then tested for BLTVA by using polymerase chain reaction after 6 to 7 wk. The leafhoppers transmitted BLTVA to seven of the eight exposed potato cultivars. Sixty-four percent of the exposed plants tested positive for the phytoplasma. In addition, 81% of the BLTVA-infected potato plants developed distinct potato purple top disease symptoms. Beet leafhoppers also transmitted BLTVA to beets and several weeds, including groundsel, *Senecio vulgaris* L.; shepherd's purse, *Capsella bursa-pastoris* (L.) Medik.; kochia, *Kochia scoparia* (L.) Schrad; and Russian thistle, *Salsola kali* L. This is the first report of transmission of BLTVA to potatoes, beets, and the above-mentioned four weed species. Results of the current study prove that the beet leafhopper is a vector of the potato purple top disease.

**KEY WORDS** *Circulifer tenellus*, insect vector, BLTVA phytoplasma, potato purple top disease

An epidemic of purple top disease of potato, *Solanum tuberosum* L., occurred in the Columbia Basin of Washington and Oregon in 2002 and caused significant yield losses and reduced tuber quality (Munyaneza 2004a,b, 2005; Munyaneza et al. 2005). The disease also was observed in subsequent years, especially in potato fields that had not been treated with insecticides (Munyaneza et al. 2005). Early investigation of the cause(s) of the disease indicated that leafhopper-transmitted phytoplasmas may have played a significant role in this disease epidemic.

The phytoplasma disease complex of potato is poorly understood. Phytoplasma-infected potato plants frequently develop foliar symptoms termed purple top, and the etiology often was attributed to the aster yellows phytoplasma as has been the case in the United States and Mexico (Hagel and Landis 1967, Banttari et al. 1993, Leyva-López et al. 2002). In 2003 and 2004, samples of potato plants showing disease symptoms were collected from potato fields throughout the Columbia Basin and tested for phytoplasmas by using polymerase chain reaction (PCR) (Lee et al.

2004a, Crosslin et al. 2005). Results indicated that all phytoplasmas detected from these potato plants belong to the clover proliferation group (16SrVI), subgroup A (16SrVI-A), and were most closely related to vinca rosette (VR), a strain of beet leafhopper-transmitted virescence agent (BLTVA). The 16S rDNA sequence analysis indicated a 99.7% gene sequence homology with VR compared with 99.2% with clover proliferation and potato witches'-broom phytoplasmas (Lee et al. 2004a). This phytoplasma also was identified as the causal agent of the dry bean phyllody disease recently observed in the Columbia Basin (Lee et al. 2004b). These results supported the view that the phytoplasma associated with the potato purple top disease in the Columbia Basin is different from the potato purple top phytoplasma reported from the north central United States and Mexico (Banttari et al. 1993, Leyva-López et al. 2002), which is related to the aster yellows group (16SrI). Thus, similar disease symptoms can be caused by two different pathogens (Crosslin et al. 2005). Sequence of a fragment from PCR products obtained from Columbia Basin phytoplasma-infected potatoes and beet leafhoppers was deposited in the GenBank data base as accession no. AY692280 (by J.M.C.). To distinguish this potato purple top phytoplasma from the aster yellows phytoplasma in Mexico and north central United States, the

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accession was identified as the "Columbia Basin potato purple top phytoplasma" and is closely related or identical to BLTVA (Munyaneza et al. 2005).

Phytoplasmas are commonly transmitted by leafhoppers (Khadhair et al. 1997, Lee et al. 1998). In 2003 and 2004, leafhoppers were collected from several sites in the Columbia Basin and identified (Munyaneza 2004a,b, 2005; Munyaneza et al. 2005). Several leafhopper species were found during the sampling and included beet leafhopper, *Circulifer tenellus* (Baker); *Macrosteles* spp.; *Ceratagallia* spp.; *Dikraneura* spp.; *Exitianus exitiosus* (Uhler); *Ballana* spp.; *Colladonus* spp.; *Amblysellus* spp.; *Paraphlepsius* spp.; *Texananus* spp.; *Balclutha* spp.; *Latalus* spp.; *Empoasca* spp.; and *Erythroneura* spp. (Munyaneza 2004a,b, 2005; Munyaneza et al. 2005). These leafhopper species were tested by PCR individually or in groups of 5–10 for the presence of the BLTVA phytoplasma (Crosslin et al. 2005). The phytoplasma was most often detected in *C. tenellus* and less frequently in *Ceratagallia* spp. All other leafhoppers tested negative for the phytoplasma, including *Macrosteles* spp., the well known vector of aster yellows phytoplasma. The association of the BLTVA phytoplasma almost exclusively with the beet leafhopper suggested that this insect species was more likely a major vector of the potato purple top disease in the Columbia Basin (Crosslin et al. 2005). However, no studies have been conducted to prove that this leafhopper actually transmits the phytoplasma associated with the potato purple top disease to potatoes and several other plants, including weeds that are found in the vicinity of potato fields and are hosts of the beet leafhopper and potential hosts to the BLTVA phytoplasma.

The work described herein was conducted to determine whether the beet leafhopper transmits the BLTVA phytoplasma to potatoes, beets, and selected weeds. In addition, we investigated development of potato purple top disease symptoms in BLTVA-infected potato plants after exposure to BLTVA-infected beet leafhoppers.

## Materials and Methods

**Sources of Potato Plants and Beet Leafhoppers.** The experiments were conducted in controlled environment rooms, greenhouse, and large outdoor cages at the USDA-ARS, Wapato, WA. Potato tubers of eight cultivars ('Russet Burbank', 'Ranger Russet', 'Umatilla Russet', 'Shepody', 'Atlantic', 'FL-1879', 'FL-1867', and 'FL-1833') used in the study were obtained from USDA-ARS, Aberdeen, ID, and CSS Farms (Dalhart, TX); FL cultivars are Frito Lay Inc. privately grown potato cultivars mainly for potato chip production. They were planted in 0.5-liter pots (Kord Products, Toronto, Ontario, Canada) in the greenhouse maintained at 26°C, 50% RH, and a photoperiod of 16:8 (L:D) h. The growth media used for potatoes consisted of a mixture of 86% sand, 13.4% peat moss, 0.5% Apex time release fertilizer (J. R. Simplot Co., Lathrop, CA), and 0.1% Micromax micronutrients (Scotts Co., Marysville, OH). The growth media pH was ad-

justed to 6.8 through the addition of dolomite lime to optimize tuber germination and growth. These plants were in seedling stage when first used in the experiment and their height ranged from 2 to 8 cm. Sugar beet, *Beta vulgaris* L. 'Saccherifera'; radish, *Raphanus sativus* L. 'Cherry Belle'; and periwinkle, *Cantharanthus roseus* (L.) G. Don 'Stardust Orchid' plants also were grown in the same greenhouse similarly to potatoes as described above; these plants also were used throughout the study.

Beet leafhoppers were originally field collected from weeds in various locations of the Columbia Basin and reared on clean sugar beet and radish plants in a controlled environment room for several generations; the room was maintained at 23°C, 50% RH, and a photoperiod of 16:8 (L:D) h. Beet leafhopper voucher specimens are deposited in the Insect Collection at the USDA-ARS, Wapato, WA.

**Source of BLTVA Phytoplasma.** Unidentified isolates of BLTVA phytoplasma used in this study were obtained from infected radish plants that were exposed to beet leafhoppers collected from various locations in Washington in spring and summer 2003; the phytoplasma in the plants was confirmed by PCR testing. The pathogen has since been maintained in our laboratory in either radish or periwinkle. Beet leafhoppers were used as vectors to inoculate phytoplasma-free plants; these leafhoppers are known to transmit BLTVA to periwinkle (Oldfield et al. 1977) and radish (Golino et al. 1989). These infected plants and leafhoppers are screened for BLTVA by PCR on a regular basis. In addition, these infected plants and leafhoppers are kept in separate controlled environment rooms from clean plants that are kept in a greenhouse room.

**BLTVA Transmission to Potatoes.** Transmission studies were conducted in summer 2005 in a room maintained at 23°C, 50% RH, and a photoperiod of 16:8 (L:D) h. After emergence, potato plants (2–8 cm in height) were transferred from the greenhouse into a 50- by 100- by 70-cm cage. The cage was made of a solid bottom, clear Mylar plastic on the front side, and organically cloth on the remaining sides. The cage was illuminated with two 120-cm fluorescent fixtures, each using two 40-W cool white tubes, suspended over the cage and ≈45 cm from the top of the plants. Before exposure, leaf tissues were taken from the plants and tested for BLTVA to ensure that plants used in the experiment were free of this phytoplasma. Two groups of 10 and 15 potato plants representing eight cultivars were exposed to ≈200 beet leafhopper adults (per cage) that had previously been reared on a mixture of BLTVA-infected radish and periwinkle plants for at least 2 mo. These two groups of potato plants were exposed to leafhoppers into the same cage at 1-wk interval. Different numbers of plants were used for each potato variety tested depending on the availability at the time of the experiment. Leafhopper adults were transferred to the transmission cage and counted using an insect aspirator. To verify that leafhoppers used in the experiment were BLTVA-infected, a sample of 10 leafhoppers was taken each time a group of

plants were exposed and tested individually for BLTVA by using PCR as described below, and the percentage of infection was determined. In addition to potatoes, three infected radish and periwinkle plants each were added to the transmission cage to provide a food source, reproduction sites, and allow nymphal development because potatoes are not the preferred host of beet leafhoppers. Furthermore, two additional tested phytoplasma-free periwinkle plants were placed in the transmission cage at the time of each potato exposure; these plants were  $\approx 1$  mo old. These periwinkle plants were removed at the same time as the exposed potato plants, maintained in the greenhouse, observed for BLTVA symptoms, and tested for BLTVA by using PCR. These periwinkle plants served to verify the infectivity of leafhoppers used in the transmission tests. After 1-wk of exposure, the potato plants were repotted into 14-liter pots (Classic Nursery Supplies, McMinnville, OR), placed outdoors in 2.5- by 2.5- by 2-m screen cages with a mesh of 175 openings per  $\text{cm}^2$  (Lumite Cage and Screen Products Inc., Gainesville, GA), treated with insecticides (methamidophos), and watered using drip irrigation; the insecticide treatments in the cages were continued during the entire experiment at 2-wk intervals to exclude potential vectors and other insects. Also, visual inspections of the presence of insects on the plants in the cages were conducted on a regular basis. In addition, eight tested clean potato plants that had not been exposed to beet leafhoppers were maintained in the outdoor cages along with the exposed potatoes to verify that no unintended transmission occurred. The plants were observed for potato purple top symptoms throughout the experimental period and were tested for BLTVA by using PCR after 6 to 7 wk.

**Inoculation of Beets and Weeds.** The experiment was conducted at the USDA-ARS, Prosser, WA. Seeds of table beets, *B. vulgaris* L. 'Detroit Dark Red'; groundsel, *Senecio vulgaris* L.; shepherd's purse, *Capsella bursa-pastoris* (L.) Medik.; kochia, *Kochia scoparia* (L.) Schrad.; prickly lettuce, *Lactuca serriola* L.; clasping pepperweed, *Lepidium perfoliatum* L.; wild radish, *Raphanus raphanistrum* L.; and Russian thistle, *Salsola kali* L. were sown in commercial potting mix (Sunshine Mix Number 1; Sun Gro Horticulture, Bellevue, WA). Plants were individually enclosed in a cage and infested with five to 10 beet leafhopper adults that had been raised on BLTVA-infected daikon radish, *Raphanus sativus* L. 'Longipinnatus'. All the plants were 10–15 cm in height when infested, except the groundsel and clasping pepperweed plants that were in rosette stage. Each cage was 40 by 40 by 60 cm and consisted of glass and 100-mesh nylon. The cages were placed in growth chambers. Some of the plants were infested twice when it seemed that most of the first group of insects did not survive for 7–10 d, at which time more insects were introduced into cages. Leafhoppers remained on the plants for 1–3 wk before the plants were sprayed with insecticide (permethrin). The plants were tested by PCR for BLTVA 5–7 wk later as described below.

**Nucleic Acid Extractions and PCR.** The nucleic acid extractions from insects, either individually or in groups of five, and plants were conducted as described by Crosslin et al. (2005). The first-round PCR reactions were performed using primer pair P1 and P7 and nested reactions used primer pair fU5 and BLTVA-int as described previously (Crosslin et al. 2005). Insects and plants were considered positive for phytoplasma if the expected amplification product of  $\approx 1.2$  kbp was visible after agarose gel electrophoresis.

## Results

Before exposure to leafhoppers, no plants used in the experiment tested positive for BLTVA phytoplasma. Beet leafhoppers readily settled on potato plants during the transmission experiments. At the two times of plant exposure, PCR results indicated that 80 and 70% of beet leafhopper adults used to inoculate potato plants, respectively, tested positive for the BLTVA phytoplasma. Also, all the clean periwinkle plants exposed at the same time as the potato plants tested positive for the phytoplasma and showed BLTVA symptoms that included floral virescence and phyllody; the symptoms were observed  $\approx 4$  wk after exposure. No insects were observed on the plants in the outdoor cages during the regular visual inspections.

None of the 16 potato plants that had not been exposed to leafhoppers and used as clean sentinel plants in the outdoor cages showed phytoplasma symptoms or tested positive for BLTVA at the end of the experiment. Of the 25 potato plants exposed to BLTVA-infected beet leafhoppers, 16 plants (64%) tested positive for the BLTVA phytoplasma, 6 to 7 wk after exposure (Table 1). In addition, 13 of the 16 BLTVA-infected plants (81%) had developed distinct potato purple top symptoms (Table 1). These symptoms included a rolling upward of the top leaves with yellowish, reddish, or purplish discoloration, leaf mottling, moderate proliferation of buds, shortened internodes, swollen nodes, aerial tubers, and early plant decline. No symptoms were observed on the plants that tested negative for the phytoplasma, 7 wk after exposure.

The beet leafhopper transmitted BLTVA phytoplasma to 10 of 11 beets and many of the weeds tested (Table 2). The phytoplasma was transmitted to at least one plant of six of the seven weed species inoculated; none of the prickly lettuce plants were positive by PCR. The beets and most of the weeds failed to produce visible symptoms of phytoplasma infection, 5–7 wk after exposure. Infected groundsel, however, developed flower abnormalities, including virescence and phyllody, typical of phytoplasma infection. A few of the kochia plants senesced prematurely.

## Discussion

Under laboratory conditions, Golino et al. (1989) successfully transmitted BLTVA to  $>40$  host plants by using the beet leafhopper as vector. The tested plants

Table 1. Transmission of BLTVA phytoplasma to eight cultivars of potatoes by the beet leafhopper

Potato cultivar	Total no. of plants exposed to leafhoppers	No. of days from initial exposure to PCR testing	No. of plants positive for phytoplasma by PCR	No. of plants with purple top symptoms
Russet Burbank	4	52	0	0
Ranger Russet	4	43	4	3
Shepody	3	43	3	3
Umatilla Russet	2	43	2	2
Atlantic	2	52	2	2
FL-1879	4	43	3	3
FL-1867	3	52	1	0
FL-1833	3	52	1	0

include several vegetable and ornamental crops previously reported as susceptible to beet curly top virus and other phytoplasmas; however, the potato and beets were not among the plants tested. Results of the current study extend the previous findings of Golino et al. (1989).

Results of the current study showed that the beet leafhopper effectively transmits the purple top disease phytoplasma to potatoes. Leafhoppers transmitted BLTVA to seven of eight potato varieties tested and to 64% of the exposed potato plants (Table 1). Also, purple top disease symptoms developed on 81% of the potato plants that tested positive for BLTVA, 7 wk after exposure to infected beet leafhoppers. Only Russet Burbank potato plants did not test positive for the BLTVA phytoplasma and did not show any purple top disease symptoms, 7 wk after exposure to leafhoppers. These observations suggest that this potato cultivar is resistant to BLTVA. However, several Russet Burbank potato plants exhibiting potato purple top disease symptoms were previously observed in the Columbia Basin and Yakima Valley in Washington and tested positive for BLTVA phytoplasma by PCR (J.E.M., unpublished). A further study to elucidate susceptibility of several different potato varieties to BLTVA, including Russet Burbank, is underway at the USDA-ARS, Wapato, WA.

The beet leafhopper is common throughout the western United States and is well adapted for life in the desert. It feeds on many cultivated crops and weed species, develops rapidly, and disperses readily to find new food sources (Hills 1937; Cook 1941, 1967; Lawson et al. 1951; Thomas 1969, 1972; DeLong 1971;

Thomas and Martin 1971; Thomas and Boll 1977; Capinera 2001). In the Columbia Basin of Washington and Oregon, this insect is very abundant in weeds near potato fields, including the ones tested in the current study, from mid-April to mid-October and has at least three generations a year (Hills 1937, Munyaneza et al. 2005). The weeds tested in the current study are very abundant in the Columbia Basin potato agroecosystem and play an important role in the survival and dispersal of the beet leafhopper. However, little is known on the role of these weeds in the epidemiology of this plant pathogen. The beet leafhopper usually moves into potato fields from weeds in mid-May to mid-June in this area and is present throughout the remainder of the growing season (Munyaneza et al. 2005). Mechanisms used by this leafhopper to effectively transmit BLTVA to potatoes are poorly understood. Previous studies indicated that the beet leafhopper does not colonize potatoes and the potato is not a suitable host plant (Radcliffe 1982, Radcliffe et al. 1993). However, results of our recent study under laboratory conditions (Munyaneza and Upton 2005) showed that when confined on potato or given no choice, the beet leafhopper survived, reproduced, and developed on this plant. Furthermore, beet leafhopper nymphs are commonly found in potato fields in Washington and Oregon (J.E.M., unpublished); however, it is not clear whether these nymphs are produced within potato fields or whether they move into potatoes from weeds in the vicinity.

In brief, results of the current study conclusively showed that the beet leafhopper effectively transmits the BLTVA phytoplasma to potatoes, beets, and some

Table 2. Transmission of BLTVA phytoplasma to table beets and selected weeds by the beet leafhopper

Common name	Scientific name	Inoculation access period (d) <sup>a</sup>	No. of plants infested	No. of plants positive by PCR <sup>b</sup>
Table beets	<i>B. vulgaris</i>	7	11	10
Kochia	<i>K. scoparia</i>	7–20 <sup>c</sup>	24	8
Russian thistle	<i>S. kali</i>	7	17	4
Prickly lettuce	<i>L. serriola</i>	7	8	0
Shepherd's purse	<i>C. bursa-pastoris</i>	7–20 <sup>c</sup>	4	4
Clasping pepperweed	<i>L. perfoliatum</i>	9–20 <sup>c</sup>	4	1
Wild radish	<i>R. raphanistrum</i>	10–15 <sup>c</sup>	18	17
Groundsel	<i>S. vulgaris</i>	7	6	4

<sup>a</sup> Plants were infested with five to 10 beet leafhoppers per plant during the indicated period before being sprayed with insecticides.

<sup>b</sup> Plants were tested by nested PCR as described in Materials and Methods, 5–7 wk after the inoculation access period.

<sup>c</sup> These plants were infested with leafhoppers twice.

weed species and supported the view that this insect is a vector of the potato purple top phytoplasma in the Columbia Basin of Washington and Oregon (Crosslin et al. 2005, Munyaneza 2005, Munyaneza et al. 2005). This is the first report of the transmission of BLTVA by the beet leafhopper to potatoes, beets, groundsel, kochia, Russian thistle, and Shepherd's purse. Further studies to elucidate the role of weeds surrounding potato fields in the dispersal of the beet leafhopper and the epidemiology of the potato purple top disease in the Columbia Basin are planned. This information is very important in designing effective management strategies to reduce incidence of the potato purple top disease and other BLTVA related diseases in affected areas by focusing monitoring and control efforts on the beet leafhopper and its wild plant hosts.

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